## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.

09/831,123

Confirmation No. 8722

Applicant Filed

Kinkade et al. August 13, 2001

Filed : TC/A.U. :

1641

Examiner

Cook, Lisa V.

For :

BIOMARKERS FOR OXIDATIVE STRESS

Docket No.

68-97

Customer No.: 23713

## DECLARATION OF JOSEPH M. KINKADE, JR. AND NGOC-ANH LE

We hereby declare as follows:

- 1. That we, together with Raymond Shapira, Peter E. Jensen, Jan Pohl and W. Virgil Brown, are inventors of the invention claimed in the above-referenced patent application.
- 2. That we have read and understand the above-referenced application, including the claims.
- 3. That we obtained hybridoma supernatants using the same procedures as given in the above-referenced specification on pages 39-46. The hybridoma supernatants were screened with performic acid oxidized OVA (PAOxOVA) and performic acid oxidized BSA (PAOxBSA) as described in the above-referenced specification on page 43 using five 96-well plates. 48 wells showed good to very high activity (range was about 5-388 mOD units/min in the kinetic assay; background was about 1 mOD unit/min). The active wells were subcloned but the experiment became contaminated.
- 4. That we carried out two additional separate and independent immunizations and fusions using the same procedure as given in the above-referenced specification on pages 39-6 and the resulting clones were designated, respectively, K1 or K2. Three clones that originated from the first fusion: K1.12.1, K1.12.6 and K1.12.H5 showed good activity (see table below). Clone K2.A12 was used as a negative control. The target was (PAoxOVA).

|          | Activity                      |
|----------|-------------------------------|
| Clone    | (mOD/min); mean of four wells |
| K1.12.1  | 50.2                          |
| K1.12.6  | 63.5                          |
| K1.12.H5 | 70.8                          |
| K2.A12   | 1.6                           |

The results in the Table below show data obtained for clones that originated from the second fusion using two different targets.

| Clone           | Target on<br>ELISA | Activity mean of 4 wells; fold increase over background |
|-----------------|--------------------|---|
| K2.F1. <u>1</u> | PAoxOVA            | 258   |
|                 | PAoxB\$A           | 593   |
| K2.F1.3         | PAoxOVA            | 630   |
|                 | PAoxBSA            | 687   |
| K2.F1.6         | PAoxOVA            | 369   |
|                 | PAoxBSA            | 514   |

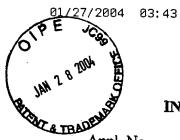
5. All statements made herein of my knowledge are true and all statements made on information and belief are believed to be true; and further, these statements are made with knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 1-27-2004

Joseph M. Kinkade, Jr.

Dated:

NGOC-ANH LE



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- 2. That we have read and understand the above-referenced application, including the claims.
- That we obtained hybridoma supernatants using the same procedures as given in the 3. above-referenced specification on pages 39-46. The hybridoma supernatants were screened with performic acid oxidized OVA (PAoxOVA) and performic acid oxidized BSA (PAoxBSA) as described in the above-referenced specification on page 43 using five 96-well plates. 48 wells showed good to very high activity (range was about 5-388 mOD units/min in the kinetic assay; background was about 1 mOD unit/min). The active wells were subcloned but the experiment became contaminated.
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| Clone    | Activity (mOD/min); mean of four wells |  |
|----------|--|--|
| K1.12.1  | 50.2                                   |  |
| K1.12.6  | 63,5                                   |  |
| K1.12.H5 | 70.8                                   |  |
| K2.A12   | 1,6                                    |  |

The results in the Table below show data obtained for clones that originated from the second fusion using two different targets.

| Cione   | Target on ELISA | Activity mean of 4 wells; fold increase over background |
|---------|-----------------|---|
| K2.F1.1 | PAoxOVA         | 258   |
|         | PAoxBSA         | 593   |
| K2,F1.3 | PAoxOVA         | 630   |
|         | PAoxBSA         | 687   |
| K2.F1.6 | PAoxOVA         | 369   |
|         | PAoxBSA         | 514   |

5. All statements made herein of my knowledge are true and all statements made on information and belief are believed to be true; and further, these statements are made with knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

| KINKADE, JR. |
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| KINKADE, JR. |
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